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Predictive and Prognostic Roles of *BRAF* Mutation in Stage III Colon Cancer: Results from Intergroup Trial CALGB 89803

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Abstract

Purpose—Alterations in the RAS-RAF-MAP2K (MEK)-MAPK signaling pathway are major drivers in colon and rectal carcinogenesis. In colorectal cancer, *BRAF* mutation is associated with

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microsatellite instability (MSI), and typically predicts inferior prognosis. We examined the effect of *BRAF* mutation on survival and treatment efficacy in patients with stage III colon cancer.

Methods—We assessed status of *BRAF* c.1799T>A (p.V600E) mutation and MSI in 506 stage III colon cancer patients enrolled in a randomized adjuvant chemotherapy trial [5-fluorouracil and leucovorin (FU/LV) vs. irinotecan (CPT11), FU and LV (IFL); CALGB 89803]. Cox proportional hazards model was used to assess the prognostic role of *BRAF* mutation, adjusting for clinical features, adjuvant chemotherapy arm and MSI status.

Results—Compared to 431 *BRAF*-wild-type patients, 75 *BRAF*-mutated patients experienced significantly worse overall survival [OS; log-rank p=0.015; multivariate hazard ratio (HR)=1.66; 95% confidence interval (CI), 1.05-2.63]. By assessing combined status of *BRAF* and MSI, it appeared that *BRAF*-mutated MSS (microsatellite stable) tumor was an unfavorable subtype, while *BRAF*-wild-type MSI-high tumor was a favorable subtype, and *BRAF*-mutated MSI-high tumor and *BRAF*-wild-type MSS tumor were intermediate subtypes. Among patients with *BRAF*-mutated tumors, a non-significant trend toward improved OS was observed for IFL vs. FU/LV arm (multivariate HR=0.52; 95% CI, 0.25-1.10). Among patients with *BRAF*-wild-type cancer, IFL conferred no suggestion of benefit beyond FU/LV alone (multivariate HR=1.02; 95% CI, 0.72-1.46).

Conclusions—*BRAF* mutation is associated with inferior survival in stage III colon cancer. Additional studies are necessary to assess whether there is any predictive role of *BRAF* mutation for irinotecan-based therapy.

Keywords

colorectal cancer; RAS; biomarker; prognosis; response; resistance

INTRODUCTION

BRAF is a part of the RAS-RAF-MAP2K (MEK)-MAPK signaling pathway. *BRAF* mutations are observed in 10-20% of colon cancers in population-based studies (1-9). In colon cancer, *BRAF* mutation is associated with proximal tumor location and microsatellite instability (MSI) (1, 3, 10-13), and with significantly worse patient survival in most (1, 6, 14-22), though not all studies (2). In contrast, MSI-high colon cancers have been associated with a significantly improved survival (1, 2, 6, 16, 23), and several studies have suggested the prognostic impact of *BRAF* mutation status may vary according to the concurrent presence or absence of MSI-high (1, 14, 21). Thus, investigation of the prognostic impact of *BRAF* mutation or MSI in colon cancer may be most informative when these markers are simultaneously assessed.

The predictive role of *BRAF* mutation in colon cancer remains less clear. Few studies have examined the impact of *BRAF* mutation on the efficacy of available chemotherapy regimens (24, 25). A recent analysis of stage III colon cancer patients enrolled in a randomized trial comparing 5-fluorouracil (5-FU) and leucovorin (FU/LV) to irinotecan (CPT11), 5-FU and leucovorin (IFL) (CALGB 89803) suggested that, among patients with MSI-high cancer, IFL conferred a superior disease-free survival when compared to FU/LV (23). In light of the association between *BRAF* mutation and MSI, we hypothesized that *BRAF* mutation in colon cancer may similarly influence the efficacy of irinotecan-based chemotherapy in this setting.

We therefore examined prognostic and predictive roles of *BRAF* mutation among stage III colon cancer patients enrolled in this National Cancer Institute (NCI)-sponsored randomized clinical trial comparing postoperative adjuvant FU/LV to IFL (CALGB 89803) (26). Since data on pathologic stage, performance status, post-operative treatment, follow-up and tumor

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molecular features such as *KRAS* and MSI status were carefully recorded in this trial, the simultaneous impact of disease characteristics and the use of adjuvant therapy could be assessed to control for potential confounding. Moreover, the simultaneous impact of *BRAF* mutational status and MSI on patient outcome could be explored.

MATERIALS AND METHODS

Study population

Patients in this study were participants in the National Cancer Institute (NCI)-sponsored Cancer and Leukemia Group B (CALGB) adjuvant therapy trial for stage III colon cancer comparing therapy with the weekly Roswell Park regimen of 5-FU and leucovorin (FU/LV) to weekly bolus regimen of irinotecan, 5-FU, and leucovorin (IFL) (CALGB 89803) (26). Between April 1999 and May 2001, 1,264 patients were enrolled on the treatment trial. Patients in the treatment trial (and thus this companion study) were eligible if they underwent a complete surgical resection of the primary tumor within 56 days prior to study entry, and had regional lymph node metastases (stage III colon cancer) but no evidence of distant metastases. Moreover, patients were required to have a baseline Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 (ambulatory) and have adequate bone marrow, renal and hepatic function. Data on family history of colorectal cancer in first-degree relatives were obtained by questionnaire at diagnosis (26). The current analysis was limited to 506 patients for whom archived formalin-fixed paraffin-embedded tumor tissue and *BRAF* sequencing data were available. All patients signed informed consent, approved by each site's institutional review board.

We compared baseline characteristics of the patients who were included in this study (with available *BRAF* data, N=506) with those who were excluded from this study due to unavailability of tissue data (N=758). We did not detect any significant or substantial difference between these two groups in terms of age, sex, body mass index (BMI), family history, tumor location, pT stage, pN stage, performance status, bowel perforation, bowel obstruction or treatment arm (all $p>0.08$). In addition, recurrence-free and disease-free survival did not significantly differ in subjects with available *BRAF* data as compared to those without *BRAF* data (multivariate HR=0.96; 95% CI, 0.79-1.18; and multivariate HR=0.95; 95% CI, 0.78-1.15, respectively).

As part of the quality assurance program of the CALGB, members of the Audit Committee visit all participating institutions at least once every three years to review source documents. The auditors verify compliance with federal regulations and protocol requirements, including those pertaining to eligibility, treatment, adverse events, tumor response, and outcome in a sample of protocols at each institution. Such on-site review of medical records was performed for a subgroup of 328 patients (26%) of the 1264 patients included in this study.

Definitions of study endpoints

The study endpoints were; (1) recurrence-free survival (RFS), defined as the time from the study enrollment to tumor recurrence or occurrence of a new primary colon tumor; (2) disease-free survival (DFS), defined as time from the study enrollment to tumor recurrence, occurrence of a new primary colon tumor, or death from any cause; and (3) overall survival (OS), defined as the time from the study enrollment to death from any cause. For RFS, patients who died without known tumor recurrence were censored at last documented evaluation by a treating provider.

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DNA extraction from tumor, *BRAF* and *KRAS* sequencing, and MSI, MLH1 and MSH2 analyses

Tumor molecular analyses were performed blinded to patient and outcome data. DNA was extracted from paraffin-embedded colon cancer tissue (27). We marked tumor areas on H&E slide, and dissected tumor tissue by a sterile needle. PCR and Pyrosequencing spanning *BRAF* codon 600 (28), and *KRAS* codons 12 and 13 were performed as previously described (27) in the laboratory at the Dana-Farber Cancer Institute. Our previous study (27) has shown that Pyrosequencing assay is more sensitive than Sanger sequencing (29), and can detect approximately 5-10% of mutant allele among a mixture of mutant and normal alleles. Microsatellite instability (MSI) was assessed by PCR for 10 markers, and MLH1 and MSH2 expression was examined by immunohistochemistry as previously described (23). Tumors with instability in ≥50% of the loci were classified as MSI-high, and those with instability in 0-40% of the loci as microsatellite stable (MSS), and the concordance between MSI testing and immunohistochemistry for MLH1 or MSH2 loss was 97% (23). For 28 cases without PCR MSI results, those with loss of MLH1 or MSH2 were classified as MSI-high, and those with intact expression of MLH1 and MSH2 as MSS. All tumor tissue analyses were performed completely blinded to data patient identity, clinical and outcome data.

Statistical analyses

The goal of this correlative study was to determine whether tumor *BRAF* mutation status was associated with clinical outcome for patients with stage III colon cancer. Patient registration and clinical data collection were managed by the CALGB Statistical Center, and analyses were conducted collaboratively between the CALGB Statistical Center and Dana-Farber Cancer Institute. All analyses were based on the study database frozen on November 9, 2009, except for the tumor *BRAF* data. All analyses used SAS version 9.2 (SAS Institute, Cary, NC) and all p values were two-sided.

The Kaplan-Meier method was used to estimate the distribution of survival time according to *BRAF* status, and the log-rank test was used to compare survival between subgroups. We used the multivariable Cox proportional hazards model to estimate survival hazard ratio (HR) by tumor *BRAF* status. The following variables were considered in the multivariable analysis: age at study entry (continuous), sex, baseline body mass index (BMI; ≥30 vs. <30 kg/m^2), family history of colorectal cancer in first-degree relatives (present vs. absent), baseline performance status (0 vs. 1-2), presence of bowel perforation or obstruction at time of surgery, treatment arm, tumor location (proximal vs. distal), pT stage (pT1-2 vs. pT3 vs. pT4 vs. unknown), pN stage (pN1 vs. pN2), *KRAS* (wild-type vs. codon 12 mutation vs. codon 13 mutation), and MSI status (high vs. MSS). A backward stepwise elimination with a threshold of p=0.20 was conducted to select covariates in the final model. pT stage was used as a stratifying variable using the strata option in the SAS “proc phreg” command. No collinearity was evident among the variables studied. Although *KRAS* and *BRAF* mutations were almost mutually exclusive (Table 1) and *KRAS* mutation overall did not influence outcome in this dataset (30), we included *KRAS* codon 12 and 13 mutations separately in the model, to examine codon-specific effects of *KRAS* mutation. The proportionality of hazards assumption was assessed using standard survival plots and by evaluating a time-dependent variable, which was the cross-product of *BRAF* and survival time (p=0.011 for RFS; p=0.22 for DFS; p=0.26 for OS). Data were missing on family history in 1% of patients, tumor location in 1% of patients, pN stage in 0.6% of patients, perforation status in 1.8% of patients, obstruction status in 0.6% of patients, and MSI status in 0.2% of patients; those were included in a majority category in multivariable Cox models to maximize the efficiency of multivariable analyses. To assess the potential differential effect of treatment arm according to *BRAF* status (or combined *BRAF* and MSI status), we performed a single multivariate Cox regression analysis, in which we could estimate the effect of treatment arm

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simultaneously in two strata of *BRAF* status (or in four strata of combined *BRAF* and MSI status) using a re-parameterization of the interaction term(s) (3). Interaction was also assessed by including the cross product of *BRAF* and another variable of interest (without data-missing cases) in a multivariate model, using the Wald test.

RESULTS

BRAF mutation in stage III colon cancer

Study participants were drawn from a multi-center study of post-operative adjuvant chemotherapy in stage III colon cancer patients who underwent a curative-intent surgical resection (CALGB 89803 protocol) (26). We included 506 cases in the current study based on availability of tumor tissue for *BRAF* sequencing, which detected c.1799T>A (p.V600E) mutation in 75 (15%) patients. This *BRAF* mutation frequency is comparable to data in the previous large population-based studies in the U.S. (1, 16). Table 1 summarizes baseline characteristics according to *BRAF* mutation status. *BRAF* mutation was significantly associated with female sex, older age, proximal tumor location, microsatellite instability (MSI)-high, and wild-type *KRAS* (all $p<0.0045$; a p value for significance was adjusted to $p=0.0045$ by Bonferroni correction).

Prognostic role of *BRAF* mutation

With median follow-up of 7.6 years among survivors, there were 183 events for recurrence-free survival (RFS) analysis, 202 events for disease-free survival (DFS) analysis, and 160 events for overall survival (OS) analysis. In a Kaplan-Meier analysis (Figure 1), *BRAF*-mutated cases experienced a non-significant trend towards inferior RFS and DFS. For *BRAF*-mutated vs. wild-type cases, 5-year RFS was 60% vs. 65%, and 5-year DFS was 55% vs. 64%, respectively. *BRAF* mutation was associated a statistically significant reduction in OS (5-year OS: 63% in *BRAF*-mutant vs. 75% in *BRAF*-wild-type; log-rank p=0.015).

In multivariate Cox regression analysis, we examined the prognostic association of *BRAF* mutation adjusting for other predictors of patient survival (Table 2). Compared to *BRAF*-wild-type cases, *BRAF*-mutated cases experienced a significantly worse OS [multivariate hazard ratio (HR)=1.66; 95% confidence interval (CI), 1.05-2.63], adjusting for other factors including MSI and *KRAS* mutational status. For RFS and DFS analyses, trends were similar in direction, but not statistically significant.

We also examined the associations of MSI and *KRAS* mutation with patient outcome. Although MSI-high tumors were independently associated with an improved OS [multivariate hazard ratio (HR)=0.61; 95% CI, 0.38-0.97], adjusting for other factors including *BRAF* and *KRAS* mutational status, *KRAS* mutations in either codon 12 or codon 13 were not associated with patient outcome.

Combined *BRAF* and MSI status, and prognosis

We further categorized patients according to both *BRAF* and MSI status to assess the joint effect on patient outcome (Table 3). Compared to patients whose tumors were both *BRAF*-wild-type and MSS (microsatellite stable), those with *BRAF*-mutated and MSS tumors experienced a trend towards an inferior OS (multivariate HR=1.61; 95% CI, 0.96-2.69). In contrast, compared to *BRAF*-wild-type MSS patients, those with *BRAF*-wild-type MSI-high tumors demonstrated consistent trends toward superior RFS, DFS, and OS. Finally, patients with *BRAF*-mutated MSI-high cancers experienced no significant difference in outcome when compared to *BRAF*-wild-type MSS patients [multivariate hazard ratio (HR)=1.02; 95% CI, 0.54-1.93], suggesting opposing prognostic effects of *BRAF* mutation and MSI-high.

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Predictive role of *BRAF* mutation for irinotecan-based therapy

We assessed the prognostic role of *BRAF* mutation within each treatment arm and the effect of treatment according to *BRAF* status. Among patients treated with FU/LV, the presence of *BRAF* mutation was associated with a significantly reduced DFS and OS (multivariate OS HR=2.43; 95% CI, 1.34-4.40) when compared *BRAF* wild-type tumors (Table 4). In contrast, among subjects treated with IFL, *BRAF* mutation was not significantly associated with patient outcome (multivariate OS HR=1.24; 95% CI, 0.67-2.31; vs. *BRAF*-wild-type).

Among patients with *BRAF*-mutated tumors, we observed a non-significant trend toward improved RFS, DFS, and OS for subjects treated with IFL when compared with FU/LV (Table 4); however, statistical power was limited and results should be interpreted with caution. Among patients with *BRAF* wild-type cancer, IFL was associated with no benefit when compared to FU/LV alone.

In a Kaplan-Meier analysis by treatment arm and *BRAF* status (Figure 1), *BRAF*-mutated cases treated with FU/LV experienced a significantly worse OS compared to *BRAF*-mutated cases treated with IFL or to *BRAF*-wild-type cases in either treatment arm (log-rank p=0.030).

Predictive role of combined BRAF and MSI subtyping for irinotecan-based therapy

We examined the predictive role of combined *BRAF* and MSI status on adjuvant treatment efficacy (Table 5). Among subjects with either *BRAF*-wild-type MSS tumors or *BRAF*-mutated MSI-high tumors, IFL was not associated with any improvement in patient outcome. Although statistical power was limited, among patients with either *BRAF*-wild-type MSI-high tumors or *BRAF*-mutated MSS tumors, IFL appeared to confer a consistent trend toward improved RFS, DFS, and OS when compared to FU/LV-treated subjects. In contrast, there appeared to be no appreciable benefit of IFL (compared to FU/LV) among *BRAF*-mutated MSI-high or *BRAF*-wild-type MSS patients.

We also performed analyses for response to IFL (vs. FU/LV) according to MSI status (Supplementary Table 1, Supplementary Figure 1) in the current dataset. There might be a possible beneficial effect of IFL in MSI-high patients, similar to the previous analysis in the CALGB 89803 trial (23).

Finally, we examined treatment effects according to status of *BRAF* mutation and MLH1 and MSH2 by immunohistochemistry (IHC) with available IHC data. There were 4 cases with MSH2 loss, and all those 4 cases were *BRAF*-wild-type and likely Lynch syndrome cases. There were 37 cases of MLH1 loss. Among those 37 cases, 17 cases were *BRAF*-wild-type and included Lynch syndrome cases. Among the 4 cases with MSH2 loss, 2 cases received IFL with no RFS, DFS or OS event (follow-time, 8.1 and 8.5 years). Among the other 2 cases with MSH2 loss in the FU/LV arm, one case experienced a RFS/DFS/OS event at 3.5 years, and the other case was censored at 6.7 years. We analyzed the effects of IFL (vs. FU/LV) in the 17 cases with MLH1 loss and wild-type *BRAF*, and multivariate HR (with 95% CI) for IFL treatment (vs. FU/LV) was 0.11 (0.011-1.08) for RFS; 0.11 (0.011-1.07) for DFS; 0.33 (0.021-5.40) for OS. These data were suggestive of good response of Lynch syndrome cases to IFL (vs. FU/LV), although statistical power was limited.

DISCUSSION

In this study of patients with stage III colon cancer participating in the randomized trial comparing post-operative IFL to FU/LV, somatic mutations in *BRAF* were associated with a statistically significant reduction in OS, with a non-significant trend toward an inferior RFS

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and DFS. These results persisted in multivariate analyses that adjusted for other predictors for patient outcome, supporting *BRAF* mutation as an independent prognostic marker in colon cancer. Furthermore, combined *BRAF* and MSI subtyping analysis suggests that *BRAF*-mutated MSS tumor is an unfavorable subtype, while *BRAF*-wild-type MSI-high tumor is a favorable subtype, and *BRAF*-mutated MSI-high and *BRAF*-wild-type MSS tumors are intermediate subtypes (Figure 1G). The independent, opposing prognostic effects of *BRAF* mutation and MSI observed in the current study is also consistent with several previous studies (6, 16-20, 22).

Interestingly, the prognostic association of *BRAF* mutation appeared to be somewhat attenuated among patients treated with IFL, whereas *BRAF* mutation was associated with a significant increase in mortality among subjects treated with FU/LV. Among patients with *BRAF*-mutated colon cancer, IFL might be associated with a non-significant trend toward improved RFS, DFS, and OS compared to FU/LV, whereas there was no apparent benefit by IFL among *BRAF* wild-type cases. However, statistical power was quite limited and caution must be taken to interpret the results. Additional studies are needed to examine the predictive role of *BRAF* mutation in colon cancer.

Although a number of studies (31-34) have assessed potential predictive roles of various genetic or tumor biomarkers for irinotecan therapy [e.g., APTX expression (31), *ABCB1* polymorphism (32), EGFR and ERCC1 mRNA expression (33)], none of these markers has yet been proven to be clinically useful. A previous analysis of patients in this clinical trial suggested that MSI-high might predict an improved patient outcome for treatment with IFL relative to FU/LV (23), although this finding was not observed in a concurrent trial conducted in Europe (35). Possibly, mismatch repair deficiency may cause DNA repair gene mutations, inhibit the DNA repair process for double strand breaks induced by irinotecan, and thereby potentiate tumor cell death (23).

Analysis of interactions between host factors (e.g., therapy) and tumor markers is increasingly important in cancer research (36, 37). A few previous studies have examined the influence of *BRAF* status on the effect of chemotherapy in colon cancer (24, 25). In the largest previous analysis, the QUASAR trial (25) observed no predictive role of *BRAF* mutation for 5-FU-based chemotherapy in stage II colorectal cancer. The MRC FOCUS trial (24) observed greater treatment effects with 5-FU plus oxaliplatin (vs. 5-FU alone) in advanced colorectal cancers with *BRAF* mutations, compared to smaller effects of 5-FU plus oxaliplatin (vs. 5-FU alone) in *BRAF*-wild-type cases. In contrast, there was a greater treatment effects on progression-free survival with 5-FU plus irinotecan (vs. 5-FU alone) in advanced colorcetal cancer with wild-type *BRAF*, compared to smaller effects on progression-free survival with 5-FU plus irinotecan (vs. 5-FU alone); however, there was no significant interaction between *BRAF* mutation and any of the treatment comparisons (24). Additional studies are necessary to assess efficacy of various treatment regimens in stage III or IV colorectal cancers. A number of studies have assessed a predictive role of *BRAF* status in targeted therapy against EGFR in stage IV colorectal cancer (38-41); *BRAF* mutation may have a predictive role for anti-EGFR therapy in monotherapy or in chemorefractory patients, but its predictive role for other settings remains to be fully determined.

Evidence suggests increased sensitivity of cells with defective mismatch repair to irinotecan (42, 43), and improved response of Lynch syndrome MSI-high cancers to 5-FU-based chemotherapy (44). On the other hand, mechanisms underlying the apparent improved outcome for patients with *BRAF*-mutated colon cancers treated with irinotecan remain speculative. *BRAF* mutation in colon cancer has been associated with high-level global DNA methylation (45) as well as widespread gene promoter methylation termed the CpG island methylator phenotype (CIMP)-high (46-49). A recent laboratory analysis found that

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increasing levels of DNA methylation substantially increased sensitivity of cancer cells to camptothecin whereas widespread hypomethylation induced resistance to camptothecin (50). Thus, responsiveness of *BRAF*-mutated cells to irinotecan may reflect increased DNA methylation associated with *BRAF* mutation. Confirmation of our observations and elucidation of the exact mechanisms underlying potential responsiveness of *BRAF*-mutated cells to irinotecan await future studies.

There are several advantages in evaluating prognostic and predictive roles of molecular biomarkers in this NCI-sponsored clinical trial of adjuvant chemotherapy. All patients had stage III colon cancer, reducing the impact of heterogeneity by disease stage. Moreover, treatment and follow-up care were all standardized within the clinical trial, and the date and nature of recurrence were prospectively recorded. In addition, detailed information on other prognostic variables was routinely collected at study entry.

We recognize that patients who enroll in randomized trials may differ from the population-at-large. To participate, patients must meet eligibility criteria, be selected as an appropriate candidate, and be motivated to participate. In addition, patients were particularly selected for this study on the basis of availability of colon cancer tissue specimens. Nonetheless, demographic data of the patients in this study did not suggest considerable selection bias. Moreover, because the study included patients from both community and academic centers across North America, our findings should reflect the general population of stage III patients in North America. In addition, although data on *BRAF* mutational status were available on a subset of patients enrolled in the trial, baseline characteristics and patient survival did not substantially differ for patients with and without available archived tumor tissue in this trial. Finally, since *BRAF* status was not available on all patients, statistical power was attenuated. As such, confirmation of our findings is clearly needed.

In conclusion, we found that *BRAF* mutation was associated with an inferior prognosis in stage III colon cancer patients, supporting tumor *BRAF* mutation as an independent prognostic biomarker in colon cancer. Although *BRAF* mutation in stage III colon cancer may possibly predict improved response to irinotecan-based chemotherapy, the predictive role of *BRAF* mutation testing remains uncertain at this time, and additional trial studies are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AJCC	American Joint Committee on Cancer
CALGB	Cancer and Leukemia Group B
CI	confidence interval
DFS	disease-free survival
5-FU	5-fluorouracil
FU/LV	5-fluorouracil and leucovorin
HR	hazard ratio
IFL	irinotecan, 5-fluorouracil and leucovorin
MSI	microsatellite instability
MSS	microsatellite stable
NCI	National Cancer Institute
OS	overall survival
RFS	recurrence-free survival

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Statement of Translational Relevance

BRAF mutation is associated with microsatellite instability (MSI) in colon cancer. Thus, the prognostic role of *BRAF* mutation or MSI in colon cancer can only be properly assessed when these markers are simultaneously determined. We examined *BRAF* mutation status in stages III colon cancer patients who enrolled in a phase III trial CALGB 89803, which randomized patients to either a combination of irinotecan, 5-fluorouracil, and leucovorin (IFL) or 5-fluorouracil, and leucovorin (FU/LV). We found that *BRAF* mutation was independently associated with inferior overall survival. We also observed a non-significant trend toward an improved overall survival of patients randomized to IFL (vs. FU/LV) among *BRAF*-mutated patients, but not among *BRAF*-wild-type patients. Our findings provide important data on the prognostic role of *BRAF* mutation. Whether *BRAF* status has any predictive role for irinotecan-based chemotherapy needs to be examined by additional studies.

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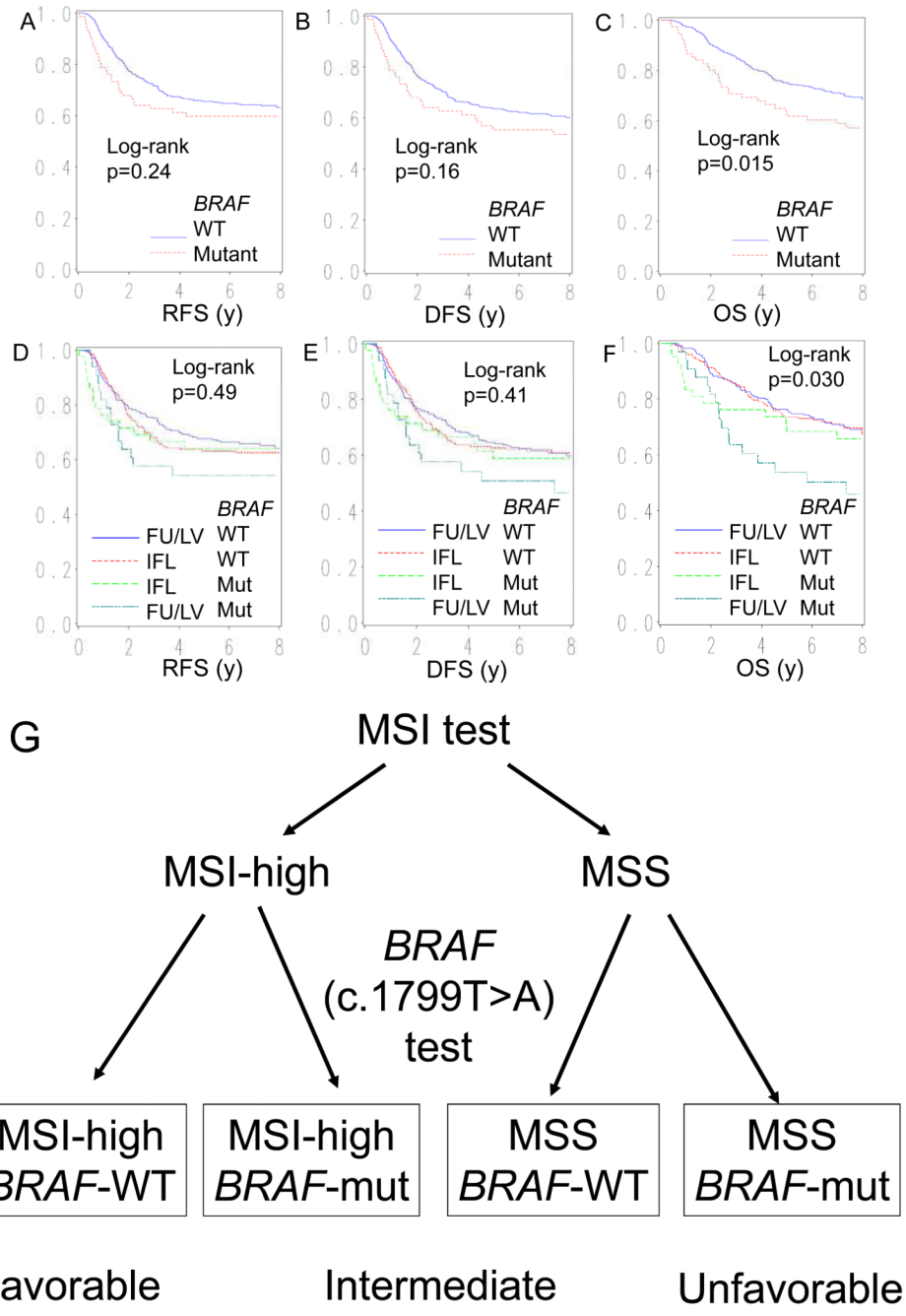


Figure 1.

BRAF mutation and clinical outcome in colon cancer.

A-C. Kaplan-Meier curves according to *BRAF* mutation in 506 stage III colon cancers for recurrence-free survival (RFS) (A), disease-free survival (DFS) (B), and overall survival (OS) (C). The y axis indicates the survival probability. D-F. Kaplan-Meier curves for RFS (D), DFS (E) and OS (F) according to treatment arm and *BRAF* mutation status. G. Proposed strategy for prognostication of colon cancer by MSI and *BRAF* tests. DFS, disease-free survival; FU/LV, 5-fluorouracil and leucovorin; IFL, irinotecan, 5-fluorouracil and leucovorin; MSI, microsatellite instability; MSS, microsatellite stable; Mut, mutant; OS, overall survival; RFS, recurrence-free survival; WT, wild-type.

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Table 1

Baseline characteristics according to *BRAF* mutational status in stage III colon cancer

Clinical or molecular feature	No. of cases	*BRAF*		P value
		Wild-type	Mutant (c.1799T>A, p.V600E)	
Total N	506	431	75	
Sex				0.0044
Male	274 (54%)	245 (57%)	29 (39%)	
Female	232 (46%)	186 (43%)	46 (61%)	
Age (years)				<0.0001
<50	99 (20%)	97 (23%)	2 (2.7%)	
50-59	131 (26%)	121 (28%)	10 (13%)	
60-69	156 (31%)	125 (29%)	31 (41%)	
≥70	120 (24%)	88 (20%)	32 (43%)	
Mean age SD	59.7 11.5	58.6 11.7	66.5 8.0	<0.0001
Body mass index (BMI; kg/m2)				0.77
<25	164 (32%)	137 (32%)	27 (36%)	
25-29	185 (37%)	159 (37%)	26 (35%)	
≥30	157 (31%)	135 (31%)	22 (29%)	
Family history of colorectal cancer in any first-degree relative				0.11
(−)	419 (84%)	361 (85%)	58 (77%)	
(+)	82 (16%)	65 (15%)	17 (23%)	
Tumor location				<0.0001
Proximal (cecum to transverse colon)	287 (57%)	219 (51%)	68 (92%)	
Distal (splenic flexure to sigmoid)	214 (43%)	208 (49%)	6 (8.1%)	
pT stage				0.29
pT1-pT2	58 (12%)	50 (12%)	8 (11%)	
pT3	409 (82%)	351 (82%)	58 (78%)	
pT4	33 (6.6%)	25 (5.9%)	8 (11%)	
pN stage				0.13
pN1	318 (63%)	277 (65%)	41 (55%)	
pN2	185 (37%)	152 (35%)	33 (45%)	
AJCC tumor stage				0.51
IIIA	48 (9%)	42 (10%)	6 (8%)	
IIIB	268 (53%)	233 (54%)	35 (47%)	
IIIC	185 (36%)	152 (35%)	33 (44%)	
III, unknown substage	5 (1%)	4 (1%)	1 (1%)	
Performance status score				0.11
0	387 (76%)	335 (78%)	52 (69%)	
1-2	119 (24%)	96 (22%)	23 (31%)	
Clinical bowel perforation				0.10
(−)	475 (96%)	407 (96%)	68 (92%)	

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Clinical or molecular feature	No. of cases	*BRAF*		P value
		Wild-type	Mutant (c.1799T>A, p.V600E)	
(+)	22 (4%)	16 (4%)	6 (8%)	
Clinical bowel obstruction				0.91
(−)	390 (78%)	333 (78%)	57 (77%)	
(+)	113 (22%)	96 (22%)	17 (23%)	
Microsatellite instability (MSI) status*				<0.0001
Microsatellite stable (MSS)	428 (85%)	387 (90%)	41 (55%)	
MSI-high	77 (15%)	43 (10%)	34 (45%)	
KRAS mutation status				<0.0001
Wild-type	330 (65%)	256 (59%)	74 (99%)	
Mutant	176 (35%)	175 (41%)	1 (1.3%)	
Treatment arm				0.10
FU/LV	267 (53%)	234 (54%)	33 (44%)	
IFL	239 (47%)	197 (46%)	42 (56%)	

(%) indicates the proportion of tumors with a specific clinical or molecular feature in *BRAF*-wild-type tumors (or *BRAF*-mutated tumors). There were cases with missing value/status for some of the variables.

AJCC, American Joint Committee on Cancer; FU/LV, 5-fluorouracil and leucovorin; IFL, irinotecan, 5-fluorouracil and leucovorin; SD, standard deviation.

*For 28 cases without MSI results by PCR, those with loss of MLH1 or MSH2 were classified as MSI-high, and those with intact expression of MLH1 and MSH2 as MSS, because concordance between MSI PCR and immunohistochemistry for MLH1 and MSH2 was very high (97%) among cases with both results available (23).

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Table 2

BRAF c.1799T<A (p.V600E), *KRAS* and MSI status and clinical outcome in stage III colon cancer

	No.	Recurrence-free survival (RFS)			Disease-free survival (DFS)			Overall survival (OS)		
		Five-year survival probability	Univariate HR (95% CI)	Multivariate HR (95% CI)	Five-year survival probability	Univariate HR (95% CI)	Multivariate HR (95% CI)	Five-year survival probability	Univariate HR (95% CI)	Multivariate HR (95% CI)
BRAF status										
Wild-type	431	0.65	1 (referent)	1 (referent)	0.64	1 (referent)	1 (referent)	0.75	1 (referent)	1 (referent)
Mutant	75	0.60	1.27 (0.86-1.87)	1.38 (0.88-2.16)	0.55	1.30 (0.90-1.88)	1.48 (0.96-2.27)	0.63	1.61 (1.09-2.37)	1.66 (1.05-2.63)
KRAS status										
Wild-type	330	0.66	1 (referent)	1 (referent)	0.64	1 (referent)	1 (referent)	0.74	1 (referent)	1 (referent)
Codon 12 mutation	123	0.64	1.03 (0.73-1.44)	1.06 (0.74-1.52)	0.61	1.04 (0.75-1.44)	1.09 (0.78-1.54)	0.76	0.89 (0.61-1.30)	0.98 (0.66-1.47)
Codon 13 mutation	53	0.67	0.81 (0.49-1.34)	0.81 (0.47-1.37)	0.66	0.82 (0.51-1.33)	0.82 (0.50-1.36)	0.75	0.83 (0.49-1.43)	0.80 (0.46-1.42)
MSI status										
MSS	428	0.64	1 (referent)	1 (referent)	0.62	1 (referent)	1 (referent)	0.74	1 (referent)	1 (referent)
MSI-high	77	0.71	0.70 (0.46-1.08)	0.56 (0.35-0.89)	0.69	0.71 (0.48-1.07)	0.57 (0.37-0.88)	0.74	0.86 (0.56-1.33)	0.61 (0.38-0.97)

CI, confidence interval; HR, hazard ratio; MSI, microsatellite instability; MSS, microsatellite stable.

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Table 3

Combined *BRAF* mutation and MSI status and clinical outcome in stage III colon cancer

***BRAF* mutation and MSI status**	**No.**	**Recurrence-free survival (RFS)**		**Disease-free survival (DFS)**		**Overall survival (OS)**	
		Five-year survival probability	**Multivariate HR (95% CI)**	**Five-year survival probability**	**Multivariate HR (95% CI)**	**Five-year survival probability**	**Multivariate HR (95% CI)**
BRAF-wild-type MSS	387	0.65	1 (referent)	0.63	1 (referent)	0.75	1 (referent)
BRAF-wild-type MSI-high	43	0.74	0.57 (0.31-1.07)	0.74	0.51 (0.27-0.95)	0.79	0.54 (0.27-1.08)
BRAF-mutant MSS	41	0.48	1.38 (0.84-2.26)	0.45	1.38 (0.85-2.25)	0.61	1.61 (0.96-2.69)
BRAF-mutant MSI-high	34	0.74	0.63 (0.32-1.28)	0.67	0.81 (0.44-1.51)	0.66	1.02 (0.54-1.93)

CI, confidence interval; FU/LV, 5-fluorouracil and leucovorin; HR, hazard ratio; IFL, irinotecan, 5-fluorouracil and leucovorin; MSI, microsatellite instability; MSS, microsatellite stable.

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Table 4

Stage III colon cancer and clinical outcome according to treatment arm and *BRAF* mutation status

	No.	Recurrence-free survival (RFS)		Disease-free survival (DFS)		Overall survival (OS)	
		Five-year survival probability	Multivariate HR (95% CI)	Five-year survival probability	Multivariate HR (95% CI)	Five-year survival probability	Multivariate HR (95% CI)
FU/LV							
BRAF-wild-type	234	0.68	1 (referent)	0.65	1 (referent)	0.76	1 (referent)
BRAF-mutant	33	0.54	1.82 (0.99-3.36)	0.51	1.83 (1.03-3.26)	0.54	2.43 (1.34-4.40)
IFL							
BRAF-wild-type	197	0.63	1 (referent)	0.63	1 (referent)	0.74	1 (referent)
BRAF-mutant	42	0.64	1.03 (0.57-1.87)	0.59	1.19 (0.68-2.11)	0.71	1.24 (0.67-2.31)
BRAF-wild-type							
FU/LV	234	0.68	1 (referent)	0.65	1 (referent)	0.76	1 (referent)
IFL	197	0.63	1.10 (0.79-1.52)	0.63	1.00 (0.73-1.37)	0.74	1.02 (0.71-1.46)
BRAF-mutant							
FU/LV	33	0.54	1 (referent)	0.51	1 (referent)	0.54	1 (referent)
IFL	42	0.64	0.62 (0.29-1.32)	0.59	0.65 (0.32-1.33)	0.71	0.52 (0.25-1.10)

The multivariate Cox regression model included the same set of covariates which were selected in the final models of the analyses in Table 2.

CI, confidence interval; FU/LV, 5-fluorouracil and leucovorin; HR, hazard ratio; IFL, irinotecan, 5-fluorouracil and leucovorin.

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Table 5

Effect of treatment arm on stage III colon cancer outcome, according to combined *BRAF* and MSI status

	No.	Recurrence-free survival (RFS)		Disease-free survival (DFS)		Overall survival (OS)	
		Five-year survival probability	Multivariate HR (95% CI)	Five-year survival probability	Multivariate HR (95% CI)	Five-year survival probability	Multivariate HR (95% CI)
BRAF-wild-type MSS							
FU/LV	212	0.68	1 (referent)	0.65	1 (referent)	0.77	1 (referent)
IFL	175	0.60	1.21 (0.86-1.70)	0.60	1.09 (0.79-1.51)	0.72	1.11 (0.77-1.61)
BRAF-wild-type MSI-high							
FU/LV	22	0.59	1 (referent)	0.59	1 (referent)	0.72	1 (referent)
IFL	21	0.91	0.24 (0.05-1.10)	0.90	0.24 (0.05-1.10)	0.90	0.30 (0.06-1.43)
BRAF-mutant MSS							
FU/LV	16	0.30	1 (referent)	0.30	1 (referent)	0.36	1 (referent)
IFL	25	0.59	0.44 (0.18-1.08)	0.55	0.52 (0.22-1.25)	0.76	0.38 (0.15-0.97)
BRAF-mutant MSI-high							
FU/LV	17	0.76	1 (referent)	0.70	1 (referent)	0.70	1 (referent)
IFL	17	0.71	1.19 (0.31-4.53)	0.65	0.97 (0.31-3.08)	0.65	0.86 (0.27-2.76)

The multivariate Cox regression model included the same set of covariates which were selected in the final models of the analyses in Table 2.

CI, confidence interval; FU/LV, 5-fluorouracil and leucovorin; HR, hazard ratio; IFL, irinotecan, 5-fluorouracil and leucovorin.